LARVAL DEVELOPMENT AND HABITS OF *LAEONEREIS CULVERI* (WEBSTER) (POLYCHAETA: NEREIDAE)[1,2]

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Laeonereis culveri (Webster) is a nereid polychaete which occurs from Connecticut to Florida, the Gulf of Mexico, Central America and the east coast of South America, where it abounds in such diverse habitats as estuaries, salt flats, sandy shoals and muddy tidal flats (Webster, 1879; Hartman, 1951; Behre, 1950; Hedgpeth, 1950; Frankenburg and Burbanck, 1963; Pettibone, 1971).

Although *L. culveri* is widely distributed, the only published account of its reproduction is by Klesch (1970) who studied a population in Texas. He found *L. culveri* to be atokous and to undergo embryonic development directly into a 3-setiger larva. He was unable, however, to rear or describe larvae beyond the 3-setiger stage and did not study larval habits.

The present account describes larval development and habits of *L. culveri* from Connecticut. It includes details on early development additional to those provided by Klesch (1970), as well as a description of later development to the adult. An attempt is made to relate observations in this study to the habitat of *L. culveri* and to the array of reproductive patterns exhibited by nereids (Reish, 1957; Clark, 1961).

MATERIALS AND METHODS

This study is based on a population of *L. culveri* inhabiting the Mystic River Estuary in eastern Connecticut. The estuary flows in a southerly direction, opening into Fishers Island Sound at 41° 19′ N, 71° 58′ W (U. S. Coast and Geodetic Chart No. 358). *Laeonereis culveri* is present at the head and upper reaches of the estuary, in the Pequotsepos tributary, and in a small cove on the western shore opposite the Mystic Seaport. The latter cove was chosen as the field site for this study since it is readily accessible and is almost completely exposed as a tidal flat at low tide. Here *L. culveri* occurs abundantly in soft sediments where individuals occupy rust-colored, mucous-lined burrows. In common with other sites populated in the estuary by *L. culveri,* the cove is characterized by extreme diurnal as well as seasonal fluctuations in salinities and temperatures. For instance, at the entrance-way to the cove, temperatures and salinities may range seasonally from 0–30° C and from < 0.5–30 ‰, respectively. These values are based on measurements taken aperiodically at different seasons and various stages of the tide from

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July, 1965 to August, 1967; temperatures were measured with a glass bulb thermometer and salinities were determined either by Barnes' (1957) method of titration with $AgNO_3$ or with a hydrometer. The broad fluctuations in salinity within the cove reflect seasonal variations in fresh water discharge from an entering stream.

Adults were readily collected from sediments taken by spade, to a depth of 10 cm, and washed with sea water on a 500 μ screen. Larvae were extracted from sediments skimmed off the tidal flat to a depth of about 2 cm, washed on a 105 μ screen, and then backwashed into a plastic dish for examination under a binocular dissecting microscope. By these methods, the population was qualitatively sampled at least once each month for the seasonal occurrence of larvae and sexually mature adults. The samples also provided worms for laboratory cultures and descriptive purposes.

Unless otherwise noted, all laboratory cultures were maintained at $22 \pm 3°$ C and in sea water with a salinity of about 30‰. The sea water was filtered, prior to use, through a Millipore prefilter pad (Millipore Filter Corporation, Bedford, Massachusetts).

Gamete-bearing worms were individually isolated in 100 cc finger bowls partially filled with sea water, but without sediments. The green alga, *Enteromorpha,* was dried, ground into a coarse powder, and introduced to the finger bowls as food (Reish and Richards, 1966). Worms established mucoid tubes along the walls of the finger bowls and readily ingested the *Enteromorpha.* Every three to four days, worms were transferred to new containers and fed a small amount of powdered *Enteromorpha.* They were also checked periodically for their state of maturity. Although they reproduced in the atokal condition, sexually mature adults were easily distinguished from immature ones by body color, the latter being brown while the former were green (Klesch, 1970). Mature oocytes were readily seen in the coeloms of females while mature males had a creamy appearance due to an abundance of sperm. Worms bearing immature gametes were present in the tidal cove from February to November, while individuals with mature gametes were available from May to October.

Neither males nor females could be induced to spawn in the presence of the opposite sex or of gametes from either sex. Males shed sperm when prodded and gently agitated, but all attempts to induce females to spawn failed. However, females spawned spontaneously, though unpredictably, within four weeks after being collected and kept at $22 \pm 3°$ C. Maintaining females at $14 \pm 1°$ C prevented them from spawning for up to ten months after being collected. In this way, mature females were occasionally held until there was a need for spawned eggs, at which time they were placed at $22 \pm 3°$ C and spawned within two weeks. Ripe males were similarly manipulated to provide sperm when needed.

Successful fertilizations were seldom attained when sperm were mixed with eggs obtained by rupturing the body of a female—even if the eggs were first washed on a fine nylon screen to remove coelomic contaminants. On the other hand, successful fertilizations occurred much more frequently when naturally spawned eggs were mixed with sperm. Mature females, therefore, were examined daily for the presence of spontaneously spawned eggs. The eggs were removed by pipette to a finger bowl containing about 300 cc of sea water. Sperm were obtained by gently prodding a male with forceps. Thus agitated, the male would swim rapidly,

lashing the rear of the body from side to side and shedding a cloudy stream of sperm from the pygidium. Three drops of sperm suspension were mixed with the eggs in the finger bowl. Time of fertilization was considered to be the time at which eggs and sperm were mixed. At 15–20 minutes after fertilization, the water was partially decanted from the finger bowl and replenished. This procedure was repeated several times to eliminate surplus sperm. Water was then changed daily until the 3-setiger larval stage had fully developed. The larvae then were removed in groups of 50 to Stender dishes containing sediment about 5 mm in depth. The sediment came either from the cove or from a nearby tidal flat and consisted of fine, flocculent particles rich in benthic diatoms which served as food. For two to three weeks prior to use in cultures, the sediment was stored in finger bowls containing Miquel's sea water medium (Needham, Galtsoff, Lutz and Welch, 1937) and placed under subdued illumination at $22 \pm 3°$ C. This treatment resulted in an increased production of benthic diatoms. Potential predators, larvae, and juveniles of *L. culveri* from the natural population were removed before adding the sediment to cultures. Every three or four days the water was removed by pipette from each larval culture and new water added. Every six or seven days, a small amount of additional sediment was pipetted into the culture.

Larval responses to various substrates were studied by using 5 cm funnels. The stems were removed from the funnels and the bases of the funnels were closed by fusing the glass with an acetylene torch. In use, a funnel was filled with sea water and the sediment to be tested was added to a depth of about 2 cm. Larvae were then added, and the funnel covered with a watch glass. The number of larvae used and the characteristics of the sediments tested are decribed later. All tests were conducted at $22 \pm 3°$ C and 30‰. The sloping walls of the funnels facilitated the observation of swimming larvae at all levels of the water column. Since a relatively small amount of sediment could be concentrated to an appropriate thickness in the bottom of the funnel, the time required to search for individuals in the sediments was minimized.

Developmental stages were examined either in hanging drops or under Saran Wrap coverslips (Dean and Hatfield, 1963). Individuals were photographed with a Polaroid camera attached to a phase contrast microscope (Dean, 1963). Outline drawings were made from photographs with the aid of a camera lucida; details were furnished from descriptions and sketches.

OBSERVATIONS AND RESULTS

Embryogenesis and early larval development

As noted by Klesch (1970), mature sperm of *L. culveri* is of a generalized type (Franzen, 1956) and the spherical pale yellow ova are demersal. At a salinity of 30‰, the latter range from 135–162 μ in diameter with a mean diameter of 147 μ.

A fertilization membrane is elevated five to ten minutes after eggs and sperm are mixed. No jelly layer is exuded. The germinal vesicle disappears and both polar bodies are extruded 10 to 15 minutes after fertilization. The first cleavage is unequal and occurs within two hours, followed by the four-cell stage at three hours and the eight-cell stage at four hours. Cleavage is spiral, and in eight to ten hours a cap of colorless transparent micromeres develops over four macromeres.
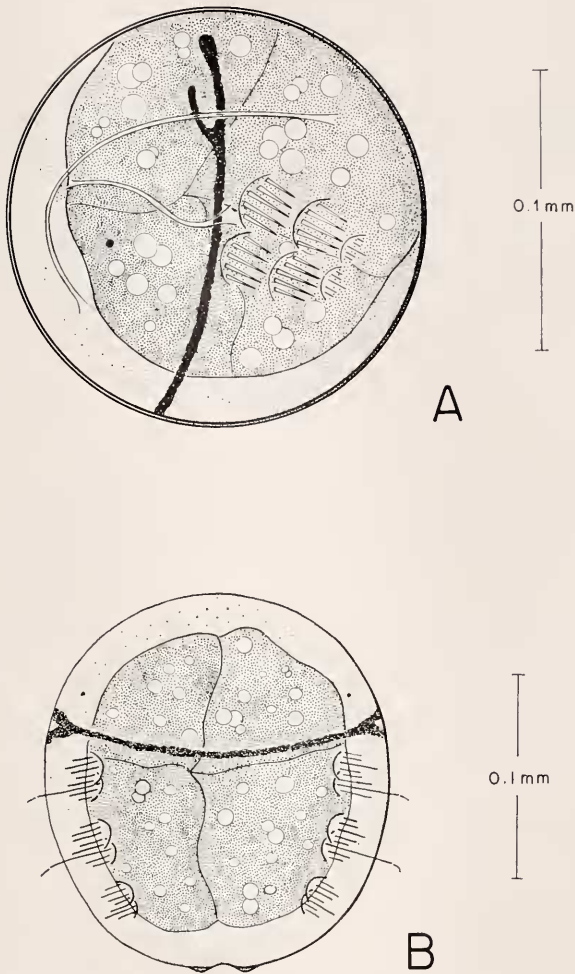
FIGURE 1. Late embryonic stages of *Laeonereis culveri:* (A) forty-eight hour, spherical, setigerous embryo in lateral view; (B) fifty-two hour, elongating embryo in ventral view.

The macromeres are yellow to amber in color and contain most of the yolk. They are unequal in size, the anterior pair being smaller than the posterior pair and the left member of the posterior pair being somewhat larger than the right one. Four macromeres usually occur, but in exceptional cases five or six may form. Embryos with more than four macromeres appear to develop normally. The micromeres completely overgrow the macromeres in typical epibolic gastrulation within 24 to 30 hours.

Further development leads directly to a spherical, setigerous embryo by 48 hours (Fig. 1A). The embryo lacks cilia and does not rotate, but does twitch sporadically. The fertilization membrane and the surface of the body are in loose contact. With each twitch, the body surface is deformed and slightly removed in places from the encompassing fertilization membrane.
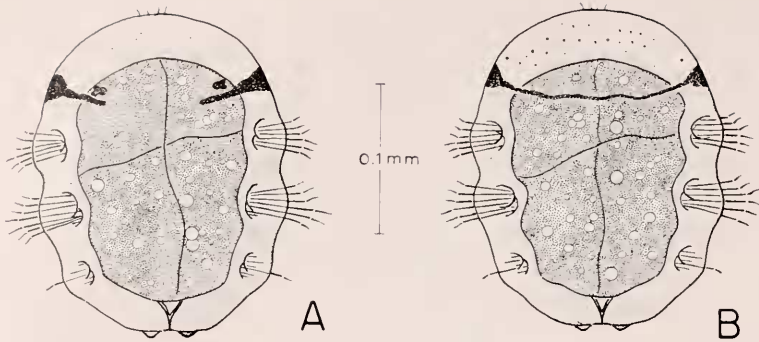
FIGURE 2.   Sixty-four hour, early 3-setiger larva of *Laeonereis culveri:*
(A) dorsal view; (B) ventral view.

Three pairs of noto- and neuropodial setal sacs develop almost simultaneously
in the setigerous embryo. The third pair of setal sacs appears to lag slightly behind
the first two pairs in its development. Each setal sac contains five or six simple
setae.

A bright red, coarsely granular band of pigment encircles the equator of the
spherical, setigerous embryo except for a broad mid-dorsal gap. The equatorial
band of pigment divides the body into a setigerous trunk region and an asetigerous
head region. All or part of this band may occasionally be absent. Two red
pigment spots are generally present on the ventrolateral surface of the head region.

The embryo begins to elongate within 52–60 hours (Fig. 1B). Twitches occur
frequently, and each twitch results in a sudden lateral compression of the body.
The fertilization membrane is now firmly attached as a cuticle to the outer surface
of the body wall. A pair of papillae, anlagen of the anal cirri, project from the
posterior extremity of the body.

As the embryo continues to elongate, each simple internal seta is transformed
into a compound homogomphous seta consisting of a shaft and serrated blade.
Setal shafts then grow outward so that the blades and parts of the shafts protrude
through the cuticle. Concurrently, rudimentary parapodial lobes form and ex-
ternally delimit the three trunk segments which correspond to the three pairs of
setal sacs. The first two pairs of parapodia develop more rapidly than the third
pair.

At 64 hours (Fig. 2A, B), all three pairs of parapodial lobes have formed, most
of the setae protrude through the cuticle, and the body now consists of a massive
head and three trunk segments. This stage of development is arbitrarily designated
as an "early" 3-setiger larva. Further development leads to a "mid" 3-setiger
larva by the third day (Fig. 3), followed by a "late" 3-setiger larva on the fourth
day (comparable to the 5-day-old larva shown in Fig. 4A, B). The 3-setiger larval
stage persists until about the seventh day, when a rudimentary fourth setiger
appears.

In the early 3-setiger larva, the equatorial band of pigment delimits the ap-
proximate posterior boundary of the head, which at this stage is not externally
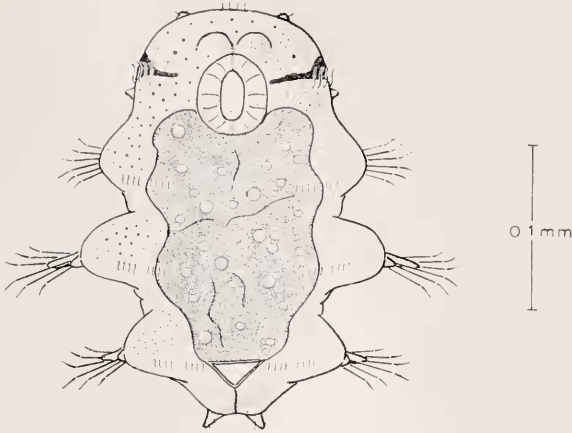
FIGURE 3.   Three-day, mid 3-setiger larva of *Laeonereis culveri* in ventral view.

demarcated from the trunk.   The head elongates and becomes sharply demarcated from the trunk in the late 3-setiger larva.

As the head elongates, the equatorial pigment-band and the pigmented spots are further removed from the trunk region.   A ventral median gap appears in the equatorial pigment-band of the mid 3-setiger larva.   The remnants of the equatorial band are gradually concentrated into a pair of lateral pigment patches in the late 3-setiger larva.   Each patch is bilobed, consisting of a large dorsolateral lobe and a smaller ventrolateral lobe.   The pigmented spots assume ventral positions and come to lie anterior to the labial palps of the late 3-setiger larva.

The simple parapodial lobes of the early 3-setiger larva elongate rapidly, and in the mid 3-setiger stage, each parapodium divides into noto- and neuroaciculate lobes.   In addition, an elongated digitate lobe appears midway between the acicular lobes of the second and third parapodia.

Two pairs of dark red eyes develop on the dorsal surface of the prostomium of the early 3-setiger larva.   The median pair of eyes is doughnut-shaped and each median eye lies in contact with a lateral cup-shaped eye.

Rudimentary cephalic appendages are present in the mid 3-setiger larva and include a pair of frontal antennae, a pair of ventrally directed labial palps, and a pair of tentacular cirri.   All of these appendages elongate to become prominent in the late 3-setiger larva.

A broad suboval pygidium is distinctly delimited from the third trunk segment in the mid 3-setiger larva, and the anal cirri continue to lengthen.

The cephalic appendages and anal cirri are clothed with sensory hairs.   Sensory hairs also project from the anterior margin of the prostomium.

A large oval pharynx with a well defined lumen is visible in the mid 3-setiger larva.   The pharynx projects posteriorly into the first trunk segment and houses a pair of simple jaws in the four-day-old, late 3-setiger larva.   At this stage, each jaw bears a terminal and a basal tooth.   On the fifth day, a weakly sclerotized tooth forms midway between the terminal and basal teeth.   Horizontal abductor-adductor movements of the jaws were first observed in the 3-setiger larva on the sixth day of
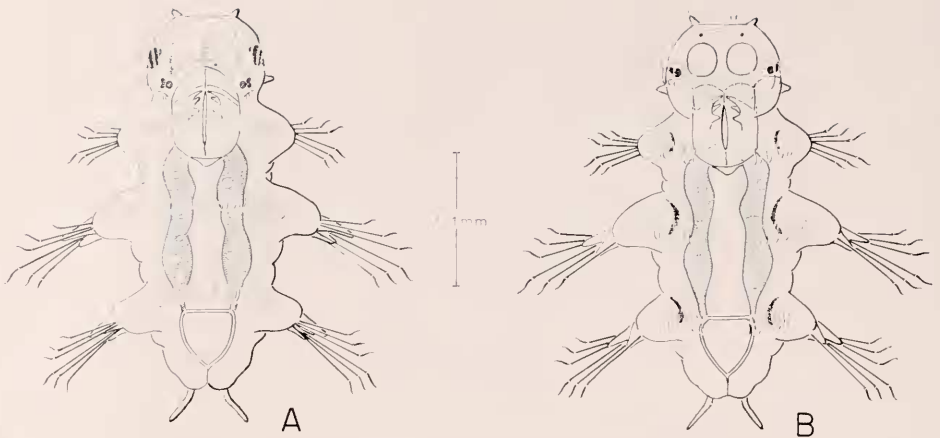
FIGURE 4. Five-day, late 3-setiger larva of *Laeonereis culveri:*
(A) dorsal view; (B) ventral view.

development. At this time, the proboscis (pharynx) exhibits weak protractor-retractor movements. Movements of the jaws and proboscis coincide with the onset of feeding.

The midgut of the early 3-setiger larva is massive and projects into the prostomium. The interior of the midgut is filled with the yolky macromeres. Gradually, the macromeres are digested and broken down to an amorphous mass of yolk that flows back and forth within the midgut of the four-day-old, late 3-setiger larva. In the five-day-old larva, the yolk is reduced to two residual masses along the wall of the midgut. A lumen occurs medially between these yolk masses, and yolk droplets flow back and forth within the lumen. On the sixth day of development, the larva feeds actively and the midgut is devoid of yolk except for an occasional yolk droplet mixed with ingested food.

The anlage of the esophagus occurs in the late 3-setiger larva as a plug-like mass of tissue projecting from the pharynx into the anterior portion of the midgut lumen.

The hindgut forms as a small blind sac posterior to the midgut of the early 3-setiger larva. The hindgut lengthens, broadens ,and becomes continuous with the midgut in the six-day-old larva.

Weak ciliary tracts form on the mid 3-setiger larva. The cilia elongate and the tracts become prominent in the late 3-setiger larva. A prototroch encircles the prostomium except for a broad dorsomedial gap. The prototroch consists of two lateral and four ventral patches of cilia.

The prostomium also bears a short patch of cilia located posterolaterad of each set of dorsal eyes (see Fig. 4A). Each ciliary patch lies within a very shallow depression which in later development deepens and constitutes the ciliated nuchal organ.

Nototrochs and gastrotrochs are present on the trunk segments. Each nototroch consists of eight patches of cilia that extend between the bases of parapodia as a dorsal transverse tract. The gastrotrochs consist of two ventral patches of cilia which extend transversely across the base of each parapodium.

The early 3-setiger larva continues the characteristic twitching movements first observed in the elongating embryo. As the parapodial lobes continue to lengthen, the twitches occur more frequently. With each twitch, the parapodia paddle back and forth; however, the larva is incapable of locomotion and remains in one position on the substrate. Likewise, the mid 3-setiger larva remains *in situ* although its body can move from side to side. Upon completion of ciliature development on the fourth day, the 3-setiger larva readily swims and crawls.

Later larval development

A fourth setiger is fully developed at eight days. Beyond this stage, growth becomes increasingly variable. In the presentation which follows, developmental changes are noted without reference to age, and the rate of growth is discussed in a later section. Also, details regarding setal and parapodial development are omitted to await later publication.

Tentacular cirri and peristomium. Four pairs of tentacular cirri eventually develop and are positioned with respect to one another, anterodorsally, posterodorsally, anteroventrally, and posteroventrally.

Tentacular cirri of the 3-setiger larva represent the definitive anterodorsal tentacular cirri. The anteroventral tentacular cirri form as budlike rudiments beneath the anterodorsal cirri in the 5-setiger larva.

The first parapodium of the 5-setiger larva acquires a medial digitate lobe which elongates considerably as the larva advances to a 7-setiger stage (Fig. 5). Upon development of the eighth trunk segment, the first parapodium is reduced to an asetigerous base supporting the elongated digitate lobe, which is now established as the posterodorsal tentacular cirrus (Fig. 6). A budlike rudiment of the posteroventral tentacular cirrus is also present. Cephalization of the first trunk segment is now complete, the latter having been incorporated into the peristomium, which bears the four pairs of tentacular cirri. Since the definitive postlarval form is established, the worm at this stage may be termed a 7-setiger juvenile as distinguished from the preceding 7-setiger larval stage.

The tentacular cirri elongate but at different rates. The posterodorsal tentacular cirri exhibit the most rapid growth and become the longest tentacular cirri in the 8-setiger juvenile. In the 20-setiger juvenile, the relative lengths of the tentacular cirri are proportionately the same as in the adult.

Ciliature. The prototroch is reduced to a few tufts of lateral cilia on the 7-setiger juvenile (Fig. 6) and is absent from the 8-setiger juvenile.

Remnants of the gastrotrochs remain on the 5-setiger larva and are absent from the 6-setiger larva.

A nototroch is acquired on each trunk segment which forms (Fig. 5), up to and including the 7-setiger juvenile stage. The nototrochal cilia are of equivalent lengths on the first four trunk segments but are progressively shorter on the fifth through eighth trunk segments. After the first trunk segment is cephalized, the first nototroch remains as a transverse ciliary band extending between the bases of the posterodorsal tentacular cirri. All nototrochs are present until the juvenile has acquired 16–18 setigers.

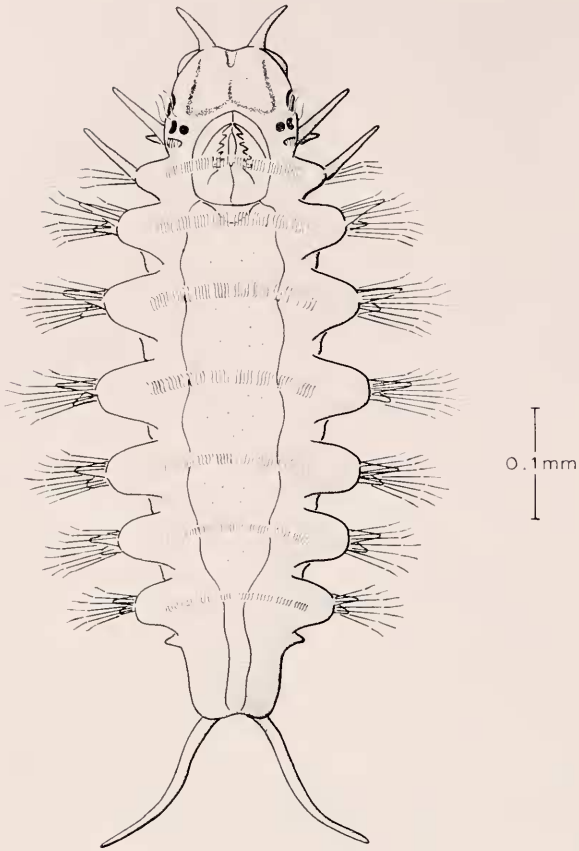The ciliated nuchal organs are retained throughout the life of the worm.

FIGURE 5.   Seven-setiger larva of *Laconereis culveri* in dorsal view.
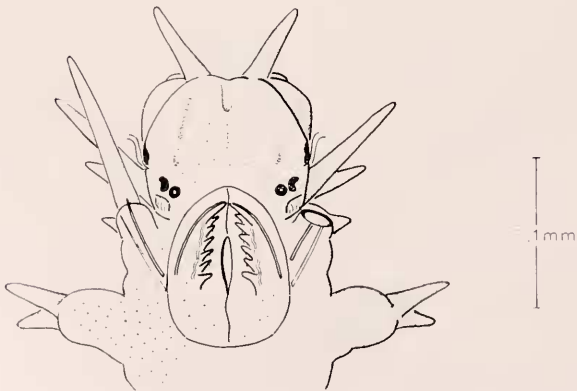

FIGURE 6.   Head and first metastomial segment of a 7-setiger juvenile *Laconereis culveri* in dorsal view; right posterodorsal tentacular cirrus removed; setae and nototroch omitted.

TABLE I

Number of teeth present on each jaw piece during different stages in the life history of Laeonereis culveri (*based on five to ten individuals examined in each life history stage*).

Life history stage (Number of setigers)	Number of teeth (Minimum and maximum)
3 (late stage, 5–6 days old)	3
4	4
5	5
6–7 setigers (7 segments)	5–6
7 setigers (8 segments)	6–7
8–12	7–8
13–14	8–9
15–16	9–10
17–29	10–11
30	11–12
Adult	12–18

Pigmentation. The red pigmented patches and spots on the prostomium gradually diminish in size; they are usually absent from the 8-setiger juvenile but may occasionally persist up to the 12-setiger stage.

Additional pigmentation forms on the five-day-old 3-setiger larva. The pigmentation appears pale yellow to white in incident light, but is black in transmitted light. There is a W-shaped band on the dorsal surface of the head and crescent-shaped bands ventrally on the bases of the first three pairs of parapodia (Fig. 4A, B). The latter bands are most prominent on the second and third pairs of parapodia. The W-shaped band is occasionally incomplete or lacking, but the crescent-shaped bands are invariably present.

The crescent-shaped bands of the first parapodia become greatly reduced in the 6-setiger larva and are absent from the 7-setiger juvenile upon cephalization of the first trunk segment. The remaining crescent-shaped bands are found up to the 15-setiger stage, while the W-shaped band may persist up to the 17-setiger stage.

Jaws and alimentary tract. Jaw development up to the 5-setiger larval stage is relatively uniform (Table I). The number of teeth present on the jaws in subsequent life history stages varies; and the definitive number of teeth is attained at about the 30-setiger stage.

The pharynx, when retracted, occupies the first trunk segment in 3- to 7-setiger larvae. The pharynx expands and elongates during later development and eventually occupies the first four metastomial segments of the adult. The pharynx of *L. culveri* is characterized by the presence of tufts of fleshy papillae instead of paragnaths. The 20-setiger stage was the earliest stage in which the papillae were observed.

The esophagus remains as a plug-like mass projecting into the anterior end of the midgut until the 12-setiger stage. During subsequent development, the esophagus elongates until it occupies the fifth to twelfth metastomial segments of the adult, with the posterior end still projecting into the midgut.
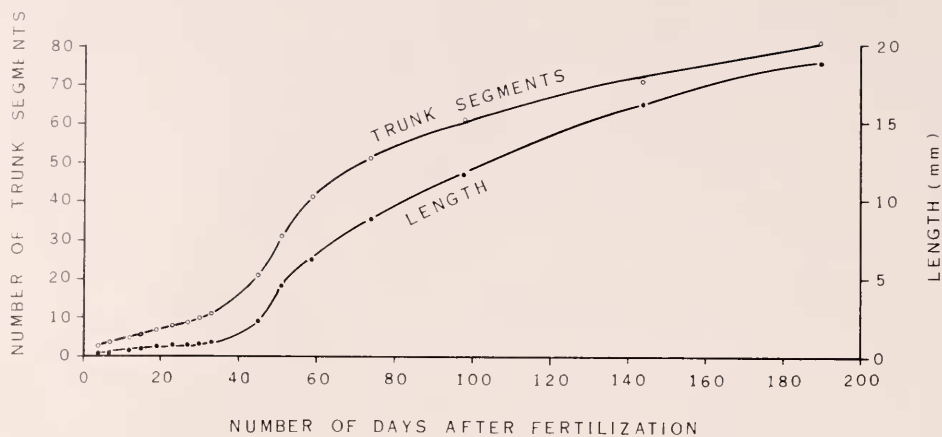
FIGURE 7.   Growth of *Laeonereis culveri* in laboratory cultures at $22 \pm 3°$ C and
30‰.   Values plotted are means of 12–24 individuals.

In worms with less than 30 setigers, the midgut can be readily distinguished
from the hindgut on the basis of pigmentation and general form.   The midgut
is yellow, relatively broad, and highly expanded ; the hindgut is colorless, relatively
narrow, highly sinuous, and sometimes doubles back on itself.   The hindgut
elongates more than the midgut.   Thus in the 30-setiger juvenile, the midgut
occupies the fifth to twelfth metastomial segments, while the hindgut occupies
the 13th to 30th metastomial segments.   Beyond the 30-setiger stage, distinction
between mid- and hindgut requires histological examination.

Growth under laboratory conditions

As noted previously, the growth of laboratory-reared worms was highly variable
beyond the 4-setiger larval stage and individual variation became progressively
greater as cultures aged.   Unfortunately, the culture techniques which were used
did not allow for careful control of the quality and quantity of food.   Therefore,
it is impossible to judge if the variability in growth was inherent or due to dif-
ferences in food conditions.   Consequently, growth of laboratory-reared worms was
followed by noting the earliest appearance of a given life history stage and its
corresponding length (Table II).   A sigmoid growth curve resulted when either
length or segment-formation was used as an indicator of growth (Fig. 7).

Thirty-two specimens (17 females, 15 males) of the F_1 generation were success-
fully reared to sexual maturity and spawned after 168–192 days of development.
The F_2 generation of worms appeared identical in morphology to those of the F_1
generation.

Sexually mature, laboratory-reared worms ranged from 16.5–20.0 mm in length,
in contrast to those from the natural population, which ranged from 28–45 mm in
length.   The lengths were taken from worms relaxed in a 0.15% solution of propy-
lene phenoxytol in sea water.

TABLE II

Development and growth of Laeonereis culveri *reared in the laboratory at 22 ± 3° C and 30‰.* All *lengths are mean values calculated from measurements of one to two dozen specimens. Postembryonic stages were relaxed in 0.15% propylene phenoxytol—sea water solution prior to being measured. Age is time after fertilization.*

Stage of development	Length (mm)	Age
Fertilization membrane elevated	—	5 minutes
Both polar bodies extruded	—	10 minutes
2-cell stage	—	1.5 hours
4-cell stage	—	3.0 hours
8-cell stage	—	4.0 hours
Early gastrula	—	8.0 hours
Late gastrula	—	24 hours
Spherical setigerous embryo	0.15	2 days
3-setiger larva (free-moving)	0.27	4 days
4-setiger larva	0.35	8 days
5-setiger larva	0.42	12 days
6-setiger larva	0.51	15 days
7-setiger larva	0.59	19 days
7-setiger postlarval stage	0.65	23 days
8-setiger postlarval stage	0.70	27 days
9-setiger postlarval stage	0.76	30 days
10-setiger postlarval stage	0.85	33 days
20-setiger postlarval stage	2.30	45 days
30-setiger postlarval stage	4.60	51 days
40-setiger postlarval stage	6.40	59 days
50-setiger postlarval stage	8.70	74 days
60-setiger postlarval stage	11.70	98 days
70-setiger postlarval stage	16.50	144 days
80-setiger postlarval stage	18.80	190 days

Benthic mode of existence

Neither eggs nor larvae were ever encountered in numerous plankton samples taken aperiodically from May to October (in 1966 and 1967) at the entranceway to the cove where *L. culveri* was very abundant. However, larvae were plentiful in all stages of development, from June to September, in the uppermost 2 cm layer of flocculent sediment on the tidal flats of the cove. Therefore, it is evident that *L. culveri* undergoes a benthic development. Further evidence supporting this view comes from laboratory observations on spawning and experiments on larval responses to sediments as detailed below.

All females which spawned in the laboratory, deposited eggs within their mucoid tubes. The spent female always remained in the tube which was irrigated by rapid dorsoventral undulations of the female's body that created a unidirectional water current and brought in sperm, whenever they were added to the water. Subsequently, the irrigational currents served to aerate the embryos until they developed to 3-setiger larvae which either crawled or swam out of the maternal tube.

Interestingly, it was only with the greatest difficulty that a spent female could be prodded out of her tube; spent males, however, were easily forced to swim out of their tubes after being prodded lightly. It was also observed that spent individuals of same or opposite sex could dwell in harmony in the same tube together

TABLE III

The effect of various sediments on the burrowing response of four-day-old 3-setiger larvae of Laeonereis culveri (*experiments terminated and results taken after a period of 12–20 hours; 50 larvae used per experiment*).

Sediment used	Number of experiments	Mean percent swimming-crawling	Mean percent burrowed in sediment	Mean percent mortality
Fine, flocculent sediment	10	2.0	97.0	1.0
Very fine to fine sands, particle diameters, 63–250 μ	5	2.8	95.6	1.6
Medium sands, particle diameters, 250–500 μ	4	95.0	4.0	1.0
Very coarse sands, particle diameters, 1000–2000 μ	5	78.8	17.2	4.0

with spawned eggs or embryos. Fighting responses or attempts to evict other occupants were never observed; indeed all life history stages appeared nonagressive.

Klesch (1970) found that spent worms die 24 hours after spawning, but in the present study they were observed to live for 10 to 16 days after spawning. Further, they continued to ingest food until one to two days prior to death. Occasionally a spent female even ingested a few eggs or embryos; the latter passed intact through the alimentary canal but became entrapped in mucoid fecal strings and decomposed. In any event, the duration of life after spawning was more than sufficient to permit the embryos to develop to freely moving 3-setiger larvae. Apparently in the natural habitat, developing eggs occur in maternal burrows and the 3-setiger larvae subsequently migrate to the surface layers of sediment. This would account for the observed absence of eggs and embryos, but the presence of larvae, in the upper 2 cm of sediments in the cove.

Whenever 3-setiger larvae were placed in a dish without sediment, they alternately swam and crawled. When light from a microscope lamp was transmitted up through the bottom of the dish, the larvae swam and crawled along the bottom. If the same light source was positioned above the surface of the water and to one side of the dish, the larvae would swim up toward the light. However, at various intervals, individual larvae returned to the bottom where they crawled toward the more shaded portions of the dish. Several minutes of crawling were again followed by swimming, and the larvae continued to alternately swim and crawl.

If an appropriate sediment was available, 3-setiger larvae rapidly entered the sediment where they formed delicate mucous-lined burrows and ceased to exhibit the swim-crawl behavior. Not all sediments elicited the burrowing response. Larvae continued to swim and crawl in the presence of medium to very coarse sands which ranged from 250–1000 μ in particle diameter (Table III). Occasionally, individuals entered interstices between the sand grains but soon emerged and resumed swimming and crawling. Larvae readily burrowed into sediments consisting of particles finer than 250 μ in diameter.

If the fine-particled, "attractive" sediments were either air-dried or ashed prior to being offered to larvae, as strong a burrowing response occurred as that elicited by the untreated sediment (Table IV). This suggests that the presence

The effect of two treatments of the flocculent, surface sediment from the natural habitat (tidal flat) on the burrowing response of five-day-old 3-setiger larvae of Laeonereis culveri *(experiments terminated and results taken after 12–16 hours; 50 larvae used per experiment).*

Treatment of sediment	Number of experiments	Mean percent swimming-crawling	Mean percent burrowed in sediment	Mean percent mortality
Untreated	4	2.5	95.5	2.0
Washed with distilled water and ashed 500° C for four hours	4	1.0	97.5	1.5
Washed with distilled water and air-dried	5	2.8	95.0	2.2

of organic films, detritus, or living food material (*e.g.*, benthic diatoms) is not required to elicit the burrowing response. The primary factor governing the burrowing response of larvae is the particle-size composition of the sediment.

As long as environmental conditions are suitable, larvae remain within their vertical burrows where they constantly move up and down. Partial emergence from the burrows occasionally occurs, followed by rapid withdrawal. While partially emerged, a larva may be observed to engulf benthic diatoms on the surface of the sediment. On one occasion, an attempt was made to feed powdered *Enteromorpha* to a culture of 3-setiger larvae residing in sediment. An excessive amount of the food was added and it soon decayed. The larvae then emerged from their burrows and swam to the surface of the water.

Four- and 5-setiger larvae retain the ability to swim by means of cilia, and their behavioral responses are identical to those of 3-setiger larvae. The cilia no longer function in swimming once the sixth setiger is formed, and later developmental stages can swim only for short distances by lateral undulations of the body. Although nototrochs do not disappear until the juvenile has acquired 16–18 setigers, these ciliary tracts function solely to create a ventilation current through the burrow and are much too weak to propel the juvenile up into the water column. However, the ventilation current is strong enough to flush fine particles of sediment and diatoms through the burrow. With loss of the nototrochs, the worm produces ventilation currents by dorsoventral undulations of the body.

Feeding habits

The gut contents of numerous specimens from the natural populations were examined. They revealed that 3- to 5-setiger larvae almost exclusively ingest benthic diatoms while older life-history stages ingest sediment and detritus.

The uppermost, flocculent layer of sediment where larvae reside on tidal flats contains an abundance of benthic diatoms. Unless larvae were provided with either fresh flocculent sediment or with sediments enriched with benthic diatoms in Miquel's medium, growth and development in the laboratory beyond the 3-setiger stage was impossible. It was also noted that if the flocculent sediment was air-dried, prior to use in larval culture, growth and survival were negligible (Table V). These observations suggest that although organic detritus may serve as food for 3- to 5-setiger larvae, it is a poor substitute for living benthic diatoms. Several

TABLE V

The effect of various substrates on survival and early growth of four-day-old 3-setiger larvae of Laeonereis culveri *after a period of eight days.*

Experimental conditions	Number of 4-setiger larvae	Number of 5-setiger larvae	Number of larvae dead	Total number
Flocculent surface sediment from natural habitat	23	23	4	50
(tidal flat); untreated; 2 cm deep	20	17	13	50
	25	20	5	50
Flocculent surface sediment from natural habitat	6	0	44	50
(tidal flat); washed with distilled water and	5	0	45	50
air-dried; 2 cm deep	10	1	39	50
Flocculent surface sediment from natural habitat	1	0	49	50
(tidal flat); washed with distilled water and	0	0	50	50
ashed for four hours at 500° C; 2 cm deep	2	0	48	50
Benthic diatoms and widely scattered particles of	0	0	20	20
sediment coating glass vessel	0	0	20	20
	1	0	19	20
	1	0	19	20
Phaeodactylum; thin layer; and no sediment	0	0	20	20
	0	0	20	20
	0	0	20	20
Glass culture dish without food or sediment	0	0	20	20
	0	0	20	20
	0	0	20	20
	0	0	20	20

attempts were made to culture larvae on benthic diatoms in the absence of sediment. The diatoms were obtained by placing flocculent sediments in culture dishes for several hours. The sediments were gently washed out, leaving numerous diatoms attached to the inner surfaces of the culture dishes. The 3-setiger larvae which were placed in such dishes fed on the diatoms; however, after a few days the larvae weakened and died. Mortalities were equivalent to those of larvae starved for the same length of time (Table V). Attempts to culture larvae on the diatom *Phaeodactylum* also failed. Thus, growth and survival of 3- to 5-setiger larvae in the laboratory required both the presence of sediment and living benthic diatoms.

Beyond the 7-setiger postlarval stage, powdered *Enteromorpha* was successfully utilized as a food source in cultures when employed with sediment. Upon reaching the 15-setiger juvenile stage, worms could be maintained solely on powdered *Enteromorpha*.

DISCUSSION

Characteristic features of a generalized nereid life cycle include (1) the structural transformation of sexually mature worms from atokal to epitokal forms that swarm near the water surface where they release gametes, and (2) a pelagic

TABLE VI

Egg sizes and modes of embryonic development of select species of nereids.

Species	Egg diameter (μ)	Ciliated embryo	Earliest larval stage	Reference
Nereis succinea	140–150	Present	Trochophore	Banse, 1954
Nicon aestuariensis	150	Present	Trochophore	Estcourt, 1966
Laeonereis culveri	135–162	Absent	3-setiger	Present study
Platynereis dumerilii	175	Present	Trochophore	Cazaux, 1969
Nereis pelagica	180	Present	Metatrochophore	Wilson, 1932
Nereis virens	170–180	Present	Trochophore	Bass and Brafield, 1972
Nereis grubei	162–380	Present	Metatrochophore	Reish, 1954
Nereis irrorata	210	?	Trochophore	Cazaux, 1969
Nereis fucata	200–250	Present	Metatrochophore	Gilpin-Brown, 1959
Nereis diversicolor	200–275	Present	Trochophore	Dales, 1950
Ceratonereis costae	200	Present	3-setiger	Durchon, 1956
Platynereis massiliensis	250	Absent	3-setiger	Hauenschild, 1951
Perinereis cultrifera	250–400	Present	3-setiger	Herpin, 1925
Nereis arenaceodentata	420–520	Absent	3-setiger	Reish, 1957

larval development, the initial stage being a lecithotrophic trochophore (Reish, 1957; Clark, 1961). However, life cycles of brackish- or freshwater-inhabiting nereids are often modified (Smith, 1958; Clark, 1961). Therefore it isn't surprising that *L. culveri*, which is a markedly euryhaline (Oglesby, 1965), estuarine species (Pettibone, 1971), reproduces in the atokal state and has a benthic larval development in which the trochophore is suppressed.

Elimination of the trochophore from the ontogeny of *L. culveri* is a consequence of the delayed development of locomotory cilia which do not appear until the 3-setiger larval stage. This contrasts with the ontogeny of most nereids in which cilia first form on the gastrula and remain on the trochophore, even if the latter is imprisoned in a jelly layer, *e.g.* as in *Perinereis cultrifera, Perinereis marioni* or *Ceratonereis costae* (Herpin, 1925; Durchon, 1956). The only nereids known to lack ciliated embryos include *L. culveri, Nereis arenaceodentata* (= *caudata*) and *Platynereis massiliensis* which are also unique in having zygotes that never produce jelly layers (Hauenschild, 1951; Reish, 1957); and in each of these species embryogenesis leads directly to a benthic 3-setiger larva, the trochophore being suppressed. The eggs of *N. arenaceodentata* and *P. massiliensis* are, however, extremely large (diameter, 250–450 μ) and give rise to unciliated, lecithotrophic larvae (the nereidogenic larvae of Hempelmann, 1911) which subsist on yolk reserves until late in development, *e.g.*, up to 17- to 19-setiger stages in *N. arenaceodentata* (Reish, 1957). On the other hand, *L. culveri* eggs are relatively small (diameter, 135–162 μ); hence yolk reserves are nearly depleted in the holotrophic 3-setiger larva which, furthermore, has the characteristic ciliary tracts of a planktogenic (Hempelmann, 1911) larva. Thus the retarded development of locomotory cilia and the resultant ontogenetic suppression of the trochophore in *L. culveri* are not associated with the production of very large, yolky eggs. This view is further verified by data in Table VI which show that there are indeed nereids with generalized modes of larval development (having pelagic trochophores or metatrochophores) whose eggs either equal or exceed in diameter those of *L. culveri*.

Although "planktogenic" and capable of swimming, the 3-setiger *L. culveri* larva typically leads a benthic existence in soft, finely particled sediments. Laboratory observations indicate, however, that if water overlying such sediments becomes "foul" (perhaps as a result of extreme organic pollution), the larva will assume a swim-crawl behavior until finding appropriately textured sediments under conditions of favorable water quality—whereupon the larva will reburrow and remain benthic. Presumably, such behavior would also be shown by larvae inadvertently flushed from their burrows by strong tidal currents. In this respect, it's noteworthy that the Berkeleys (1953) observed 3-setiger larvae of *Micronereis nanaimoensis* remaining pelagic for two to three weeks unless encountering a sandy substrate into which they burrowed and left only if agitated. Other "planktogenic" 3-setiger larvae known to be benthic are those of *Ceratonereis costae* (Durchon, 1956) and *Nereis virens* (Bass and Brafield, 1972; personal observations).

Clearly, the possession of planktogenic attributes does not imply that a necto-chaetous nereid larva is pelagic. As in *L. culveri*, larval ciliature may function primarily in ventilating burrows and only secondarily in swimming. Indeed, *L. culveri* is apparently unique in having segmental ciliary tracts on each of the first eight trunk-segments and in retaining these tracts for the purpose of burrow ventilation until about the 16-setiger stage when undulatory body movements commence to create ventilating currents. Segmental ciliary tracts of other nereids seldom number more than four, as in *Nereis japonica* (Izuka, 1908) and are lost early in larval life, *e.g.* at the 6-setiger stage of *Nereis fucata* (Gilpin-Brown, 1959).

The reproduction and development of *L. culveri* in Connecticut is generally similar to that reported by Klesch (1970) for the same species in Texas, except for the following details: 1) spawning occurs in Texas from August to September and January to February (temperatures, 15.0–33.9° C; salinities, 28.7–54.0‰); this contrasts with the single spawning season in Connecticut, from June to September (temperatures, 20.5–27.0° C; salinities, 14.8–30.2‰); Connecticut worms were inhibited from spawning in the laboratory at $14 \pm 1°$ C which approximates the minimum seasonal spawning temperature in Texas; 2) unlike in Connecticut, worms in Texas rarely shed gametes spontaneously and fertilization of stripped gametes proved highly successful; 3) Texas worms died 24 hours after spawning (apparently at 22° C; salinity not reported); Connecticut worms survived 10–16 days after spawning (at $22 \pm 3°$ C; 30‰); 4) diameters of mature eggs in Texas *vs.* Connecticut were 150–200 μ and 135–162 μ, respectively; 5) time of development, from fertilized egg to late 3-setiger larva, was seven days in Texas (at 22° C; salinity not reported) but only four days in Connecticut ($22 \pm 3°$ C; 30‰); and swim-crawl movements commenced in five-day-old, early 3-setiger Texas larvae but did not occur until the late 3-setiger stage in Connecticut.

The presence of warmer seasonal temperatures in Texas may promote biannual spawning, while in Connecticut spawning is restricted to summer months when temperatures exceed 20° C. Additional study is needed to determine the significance of these as well as other geographic differences in the reproduction and development of *L. culveri*. Further, a geographic comparison of development can presently be made only up to the 3-setiger larval stage since Klesch (1970) did not describe later laval development.

Summary

The nereid polychaete *Laeonereis culveri* (Webster) reproduces in the atokal condition in the Mystic River Estuary, Connecticut. Females spawn demersal eggs, 135–162 μ in diameter, that do not extrude a jelly layer upon fertilization and that give rise to unciliated embryos. Embryogenesis leads directly to a 3-setiger larva in four days (at $22 \pm 3°$ C; 30‰). The trochophore is suppressed due to the retarded development of locomotory cilia which do not appear until the 3-setiger larva has formed.

Although provided with ciliary tracts and capable of swimming, *L. culveri* larvae are normally benthic in habit. They reside in burrows within the upper 2 cm of fine, flocculent sediments where they feed chiefly on benthic diatoms until developing past the 5-setiger stage after which they become non-selective deposit feeders. The primary function of the larval ciliature is to pass water currents through the burrow. In the laboratory, 3- to 5-setiger larvae alternately swim and crawl in the absence of sediments or in the presence of coarse sediments (particle diameters $> 250 \mu$); but they readily burrow into and remain within fine sediments (particle diameters $< 250 \mu$) even if the latter are relatively free of organic matter. Swim-crawl behavior also appears to be elicited under conditions of unfavorable water quality. When the sixth setiger forms, the larva is no longer capable of swimming by means of its cilia.

Development beyond the 3-setiger stage approximates that of other species except that *L. culveri* larvae are apparently unique in ultimately developing eight nototrochs (one per each of the first eight trunk segments). The nototrochs are retained and function in burrow ventilation until the 16- to 18-setiger stages when body undulations commence creating ventilation currents.

Larval development is completed with addition of the eighth trunk segment. At this stage, the tentacular segment (first trunk segment) is incorporated into the peristomium and the first pair of parapodia are modified to form the posterodorsal tentacular cirri.

Sigmoid growth curves resulted when either length or segment-formation was used as an indicator of growth among laboratory-reared worms. Sexually mature, laboratory-reared worms were only about one-half the length of sexually mature worms in a natural population. Thirty-two individuals (17 females, 15 males) of an F_1 generation were reared to maturity and spawned after 168–198 days of development. F_2 generation larvae were identical in morphology to those of the F_1 generation.

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